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Effects of antihypertensive therapy on intrarenal angiotensin and bradykinin levels in experimental renal insufficiency

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Background. Whereas angiotensin converting enzyme inhibitors and angiotensin type 1 receptor antagonists have beneficial effects in the remnant model of renal failure, calcium channel blockers do not consistently improve renal disease in this model. This study examined whether these different means of blood pressure reduction have different effects on renal levels of angiotensin (Ang) and bradykinin peptides.

Methods. Rats subjected to five-sixths nephrectomy were divided into groups with similar hypertension and proteinuria at 4 to 5 weeks. They then received either no treatment, or enalapril, losartan or nifedipine for 2 weeks. Following repeat measurements of proteinuria and blood pressure, Ang II and bradykinin peptides were measured in the remnant kidney and renin, Ang II, and aldosterone were measured in the plasma.

Results. All three drugs had equivalent blood pressure-lowering effects. Enalapril and losartan reduced proteinuria but nifedipine did not. Reduction of proteinuria in rats treated with enalapril and losartan was associated with a reduction in Ang II levels in both the peri-infarct and intact portions of the remnant kidney. By contrast, nifedipine increased Ang II levels in the intact portion of the remnant kidney. Losartan reduced bradykinin levels in the peri-infarct portion of the remnant kidney while enalapril reduced bradykinin levels in the intact portion of the remnant kidney. Nifedipine had no effect on intrarenal bradykinin levels.

Conclusions. The differential effects of enalapril, losartan and nifedipine on proteinuria and intrarenal Ang II and bradykinin levels suggest that the ability of an antihypertensive to decrease proteinuria may depend on its ability to decrease kidney Ang II and bradykinin levels.

Whereas effective control of hypertension slows the decline of renal function in progressive renal disease [1], some antihypertensive agents offer more effective protection than expected from blood pressure reduction. Angiotensin-converting enzyme (ACE) inhibitors slow the progression of renal disease to a greater extent than alternate antihypertensive treatment despite equivalent blood pressure reduction [2]. Moreover, the equivalent effects of angiotensin (Ang) II type 1 (AT_1) receptor blockade and ACE inhibition in preventing renal disease in animal [3, 4] and human studies [5, 6] suggest an important role for Ang II in the pathogenesis of renal disease [7]. In contrast, despite effective blood pressure control, calcium channel blockers do not consistently slow the decline of renal function or decrease proteinuria in human studies [8–10].

Results obtained in rats subjected to renal ablation, for the most part, are consistent with those obtained in human studies. Converting enzyme inhibition and AT_1 receptor antagonists repeatedly have been shown to normalize blood pressure, reduce proteinuria and prevent glomerular injury in this model [3, 7, 11]. In contrast, calcium channel blockade generally has been found to have less if any beneficial effect [12–14]. A number of reasons have been proposed for the lack of beneficial effect of calcium channel blockade, including altered autoregulation of the afferent arteriole, inconsistent blood pressure reduction and failure to modify the intrarenal effects of Ang II [13, 15]. The goal of the current study was to determine if the effects of different antihypertensive agents on proteinuria in the renal ablation model could be related to their effects on intrarenal Ang II and bradykinin levels. We compared the effects of enalapril, an ACE inhibitor, with losartan, an AT_1 receptor antagonist, and nifedipine, a dihydropyridine calcium channel antagonist, on renal peptide levels and circulating components of the renin-angiotensin-aldosterone system (RAAS).

METHODS

Male Munich Wistar rats weighing 250 to 300 g were subjected to partial renal ablation by removal of the right kidney and ligation of the arterial branches supplying 2/3 of the left kidney as previously described [16]. All animal

Key words: angiotensin II, bradykinin, renin, hypertension, renal failure, proteinuria, remnant kidney, losartan, nifedipine.

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experiments were carried out according to protocols approved by the institutional animal care committee. Two experiments were performed. In the first, enalapril treatment was compared to losartan or no treatment. Five weeks after reduction of renal mass, rats were divided into three groups with similar values for body weight, systolic blood pressure, and proteinuria. Group 1 ($N = 7$) continued to receive no treatment while Group 2 ($N = 9$) received enalapril 50 mg/L in drinking water and Group 3 ($N = 9$) received losartan 180 mg/L in drinking water. Blood pressure and proteinuria were measured again after two weeks and rats were then sacrificed for measurements of plasma renin, angiotensin peptides and aldosterone, as well as kidney angiotensin and bradykinin peptides. In the second experiment nifedipine treatment was compared to enalapril or no treatment. Four weeks after renal mass was reduced, body weight, systolic blood pressure, and proteinuria were measured and the rats divided into three treatment groups: Group 1 ($N = 17$) was untreated; Group 2 ($N = 16$) received enalapril 25 mg/L in drinking water; Group 3 ($N = 17$) received the long acting formulation of nifedipine (Bayer Corporation, Tarrytown, NY, USA) 0.07% mixed in powdered rat chow. The dose of nifedipine required to lower blood pressure to an equivalent degree as enalapril was determined in a pilot experiment. Because of the requirement to administer nifedipine in powdered rat chow, in the second experiment all animals were placed on a normal powdered rat chow diet five days before being divided into treatment groups and were maintained on powdered rat chow during the treatment period.

Rats in all groups were killed by decapitation at the close of the study. Trunk blood was collected and the kidneys were then rapidly removed for tissue hormone analysis. Remnant kidneys were cut with a razor blade into three weighed pieces prior to analysis. One piece, denoted the "intact portion," included only tissue distant from the infarct borders. A second piece (denoted the "peri-infarct portion") was cut to include the infarct and its borders. The third piece consisting of the remaining kidney tissue was discarded. The purpose of dividing the remnant kidney was to separate areas of high and low renin content as described by Correa-Rotter et al [17]. In the first experiment, angiotensin and bradykinin peptides were measured in the kidney and plasma renin activity (PRA), and the angiotensin peptides and aldosterone were measured in the plasma. In the second experiment two cohorts of animals were used. In the first cohort, angiotensin and bradykinin peptides were measured in the kidney and PRA, angiotensin peptides and aldosterone were measured in the plasma. In the second cohort, renin content was measured in the kidney.

Systolic blood pressure was determined as the mean of five individual measurements using the tail cuff method. Urine protein excretion was determined as the mean of measurement on two non-consecutive 24-hour urine collections assayed with the Coomassie blue method. Blood pressure and urine protein measurements were not made on the day prior to decapitation in order to reduce stress on the animals.

Measurement of angiotensin and bradykinin peptides

Pieces of fresh kidney were immediately homogenized in 10 mL of 4 mol/L guanidine thiocyanate/1%-trifluoroacetic acid. After centrifugation the supernatant was applied to Sep-Pak C18 cartridges (Waters Chromatography, Milford, MA, USA) and extraction and subsequent processing performed as described previously [18]. The dried angiotensin and bradykinin peptides were acetylated and treated with piperidine before high performance liquid chromatography and subsequent N-terminal directed radioimmunoassay (RIA). Data were corrected for recovery as reported previously [19]. Trunk blood for plasma Ang I and Ang II was collected into tubes containing 0.5 mL inhibitor solution [1 mmol/L renin inhibitor, acetyl-His-Pro-Phe-Val-Sta-Leu-Phe-NH_2 [20], 146 μmol/L pepstatin, 50 mmol/L 1,10 phenathroline, 125 mmol/L ethylenediaminetetra-acetate (EDTA), 2 g/L neomycin sulfate, 2% dimethyl sulfoxide (DMSO), and 2% ethanol]. After centrifugation the plasma was immediately extracted with Sep-Pak C18 cartridges and dried samples were processed as described for the kidney samples.

Measurement of PRA and aldosterone

Trunk blood was collected into heparinized tubes, centrifuged at 1600 × g and stored at −40°C until analysis. Ten microliters of 0.5 mol/L EDTA, 8 μg of dimercaprol and 0.3 mg of 8 hydroxyquinoline were added to 100 μL of plasma sample before PRA measurement to inhibit angiotensinases. PRA was measured by the generation of Ang I after three hours incubation at 37°C and subsequent RIA using a commercially available kit (NEN Life Sciences, Boston, MA, USA). Aldosterone was measured by RIA using a commercially available kit (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA, USA). Kidneys for renin analysis were cut into peri-infarct and intact portions as described above. They were then homogenized in a protease-inhibiting buffer containing EDTA, dimercaprol, phenylmethylsulfonyl fluoride (PMSF), 8-OH-quinoline sulfate and ammonium acetate. Kidney renin concentration was measured in the supernatant of the kidney homogenates after performing three freeze thaw cycles and a 1:1000 dilution with protease inhibiting buffer. Aliquots of diluted tissue homogenate were then incubated at 37°C for two hours with excess renin substrate in the form of nephrectomized rat plasma, and Ang I generation was quantified by RIA. Values are expressed per gram of kidney tissue.

Table 1. Body weight (BW), systolic blood pressure (SBP), and proteinuria ($U_{pro}V$) in remnant rats before and after 2 weeks of antihypertensive treatment (Rx)

	BW Pre-Rx	BW Post-Rx	SBP Pre-Rx	Final SBP	$U_{pro}V$ Pre-Rx	$U_{pro}V$ Post-Rx
	g		*mm Hg*		*mg/day*	
Experiment 1						
Untreated $N=7$	292 ± 7	294 ± 8	193 ± 3	197 ± 9	152 ± 22	184 ± 16
Enalapril $N=9$	287 ± 4	301 ± 1	183 ± 6	142 ± 5[a,c]	125 ± 15	60 ± 10[a,c]
Losartan $N=9$	280 ± 7	293 ± 5	189 ± 7	125 ± 6[a,c]	170 ± 25	76 ± 13[a,c]
Experiment 2						
Untreated $N=17$	274 ± 3	287 ± 6	205 ± 5	217 ± 6	123 ± 20	152 ± 17
Enalapril $N=16$	270 ± 4	290 ± 6	200 ± 7	156 ± 3[a,c]	124 ± 13	72 ± 9[a,c]
Nifedipine $N=17$	276 ± 5	295 ± 8	192 ± 7	146 ± 5[a,c]	115 ± 15	181 ± 20[b,c]

Values are mean ± SEM.
[a] $P < 0.05$ vs. untreated
[b] $P < 0.05$ vs. enalapril
[c] $P < 0.05$ vs. before treatment

Statistical analysis

Data are presented as means ± SEM. Statistical analysis was performed by analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) test. Parameters measured before and after treatment and between peri-infarct and intact portions of each treatment group were compared using the paired *t* test. Logarithmic transformation of the data was performed when required to obtain similar variances among groups. Two-way ANOVA was used to assess the effect of enalapril on bradykinin levels between the two experiments. The software package Statview 5.0 (Abacus Concepts, Inc., Berkeley, CA, USA) was used for analyses. A *P* value of <0.05 was considered statistically significant.

RESULTS

Blood pressure, proteinuria, body weight and plasma creatinine

Body weight, systolic blood pressure, and proteinuria are presented in Table 1. Body weight, systolic blood pressure and urinary protein excretion were matched in the treatment groups before drug administration. Treatment with enalapril, losartan or nifedipine did not affect body weight, which was not significantly different from untreated controls in both experiments. As expected, rats subjected to renal ablation developed hypertension and proteinuria. In experiment 1, treatment with either enalapril or losartan caused a reduction in blood pressure that was not significantly different between the two groups. In experiment 2, treatment with enalapril or nifedipine caused similar reductions in blood pressure. In both experiments, reduction of blood pressure with enalapril was associated with significant reduction in proteinuria. Losartan treatment was associated with a significant reduction in proteinuria, similar to that seen with enalapril. In contrast, in experiment 2 the reduction of blood pressure with nifedipine was accompanied by a significant rise in urinary protein excretion.

Plasma renin, aldosterone, and angiotensin peptides

Measurements of circulating renin-angiotensin-aldosterone components are summarized in Table 2. In experiment 1, enalapril and losartan caused similar increases in PRA and Ang I levels. Losartan, but not enalapril, increased plasma Ang II levels and enalapril, but not losartan, decreased the plasma Ang II/Ang I ratio, which provides an index of the rate of conversion of Ang I to Ang II. Neither treatment altered aldosterone levels. In experiment 2, enalapril and nifedipine increased PRA levels. Enalapril, but not nifedipine, caused a significant increase in plasma Ang I levels and a reduction in the plasma Ang II/Ang I ratio. Neither drug significantly altered plasma levels of Ang II or aldosterone.

Kidney renin concentration

Kidney renin concentration was measured in experiment 2. The concentrations of renin observed in the intact and peri-infarct portions of the remnant kidney are depicted in Figure 1. The renin content in the peri-infarct portion was higher by severalfold than in the intact portion of the kidney in the enalapril and untreated groups. Treatment with either enalapril or nifedipine did not significantly increase it further. Both enalapril and nifedipine increased renin content in the intact portion of the remnant kidney.

Kidney angiotensin peptides

Angiotensin I and Ang II peptides and the Ang II/Ang I ratio in the intact and peri-infarct portions of the remnant kidney for both experiments are presented in Figure 2. Ang I levels were higher in the peri-infarct than in the intact portion of the remnant kidney of all groups for both experiments, and Ang II levels were higher in the peri-infarct portion in the untreated and nifedipine treated rats of experiment 2. Enalapril had similar effects on kidney angiotensin peptide levels in experiments 1 and 2. Both enalapril and losartan reduced

Table 2. Components of the circulating renin angiotensin aldosterone system after two weeks of antihypertensive treatment

	PRA	Aldosterone	Ang I	Ang II	Ang II/Ang I ratio
	ng Ang I/mL/hr	*pg/mL*	*fmol/mL*		*fmol/fmol*
Experiment 1					
Untreated $N = 5$–7	0.3 ± 0.1	116 ± 38	3 ± 1	10 ± 3	5.6 ± 1.6
Enalapril $N = 6$–9	10 ± 3^{a}	154 ± 27	62 ± 15^{a}	4 ± 1	0.2 ± 0.1^{a}
Losartan $N = 6$–9	10 ± 2^{a}	174 ± 41	69 ± 24^{a}	$155 \pm 32^{a,b}$	3.9 ± 0.9^{b}
Experiment 2					
Untreated $N = 8$–9	0.8 ± 0.1	140 ± 46	5 ± 1	16 ± 7	4.0 ± 0.8
Enalapril $N = 9$–10	8.3 ± 2.7^{a}	113 ± 47	55 ± 13^{a}	39 ± 21	0.8 ± 0.4^{a}
Nifedipine $N = 10$–11	4.2 ± 1.4^{a}	328 ± 214	10 ± 4^{b}	12 ± 2	2.9 ± 1.3^{b}

Values are mean ± SEM. Abbreviations are: REM, remnant; PRA, plasma renin activity; Ang, angiotensin.
[a] $P < 0.05$ vs. untreated
[b] $P < 0.05$ vs. enalapril

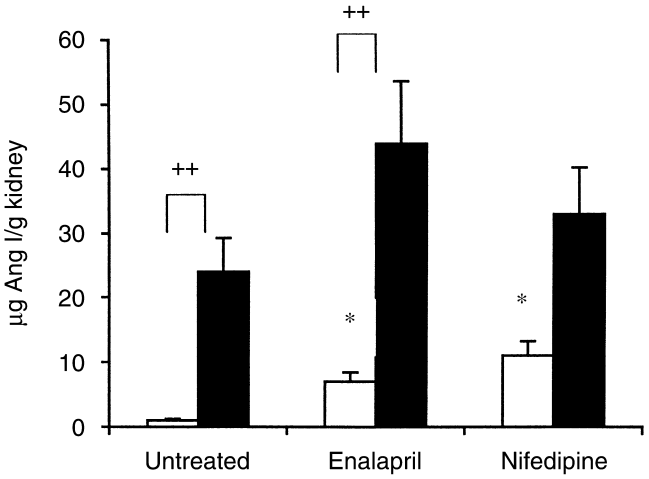

Fig. 1. Kidney renin concentration in experiment 2. Symbols are: (□) intact portion of the remnant kidney; (■) peri-infarct portion of the remnant kidney. *$P < 0.05$ vs. corresponding region of untreated rats; ++$P < 0.01$ peri-infarct vs. intact portion. Data are shown as mean ± SEM and $N = 6$ to 8 in each group.

Ang II levels in both the peri-infarct and intact portions of the remnant kidney. Neither drug affected Ang I levels in the kidney, whereas both drugs reduced the Ang II/Ang I ratio in the peri-infarct portion. Losartan also reduced the Ang II/Ang I ratio in the intact portion. By contrast, nifedipine increased Ang II levels in the intact portion to a level significantly greater than both the untreated and enalapril treated rats. Ang I levels were not altered and there was an increase in the Ang II/Ang I ratio in the intact portion with nifedipine treatment.

Kidney bradykinin peptides

The concentration of BK (1-9) and its metabolite BK (1-7) observed in renal tissue are shown in Figure 3. The BK (1-7)/BK (1-9) ratio gives an indication of the rate of metabolism of BK (1-9) to BK (1-7) by ACE and other peptidases including neutral endopeptidase. Both BK (1-7) and BK (1-9) levels were higher in the peri-infarct than in the intact portion of the remnant kidney for the untreated, enalapril, and losartan treated rats of experiment 1. However, the levels of BK (1-7) and BK (1-9) in the intact portion were much higher in experiment 2 than in experiment 1, with a smaller difference in bradykinin peptide levels between peri-infarct and intact portions in experiment 2. Enalapril reduced BK (1-9) levels in the peri-infarct portion in experiment 1, but the effects of enalapril on kidney kinin levels were not statistically significant in experiment 2. When the effects of enalapril in experiments 1 and 2 were analyzed together by two-way ANOVA, enalapril reduced BK (1-9) levels ($P = 0.02$) in the intact portion, and increased the BK (1-7)/BK (1-9) ratio ($P = 0.03$) in the peri-infarct portion of the remnant kidney. Losartan reduced BK (1-7) levels in the intact portion and both BK (1-7) and BK (1-9) levels in the peri-infarct portion, associated with an increase in the BK (1-7)/BK (1-9) ratio in the peri-infarct portion of the remnant kidney. By contrast, nifedipine did not modify renal bradykinin levels.

DISCUSSION

In accord with previous studies, enalapril and losartan lowered blood pressure and proteinuria in rats subjected to renal ablation. Nifedipine treatment, despite an equivalent blood pressure lowering effect, did not reduce proteinuria. The different effects of the drugs on proteinuria were associated with differences in their effects on intrarenal peptide levels. Enalapril and losartan reduced renal Ang II levels while nifedipine increased these levels. In addition, losartan, and to a lesser extent enalapril, reduced intrarenal bradykinin levels. These findings support a primary role for Ang II in remnant glomerular injury, and suggest further that Ang II may stimulate increased activity of the intrarenal kallikrein kinin system in the remnant kidney. Moreover, these data suggest that bradykinin may mediate in part the actions of Ang II in this condition.

Our findings regarding the effects of nifedipine and enalapril on remnant glomerular injury are consistent with those of Griffin, Picken and Bidani [14]. They showed

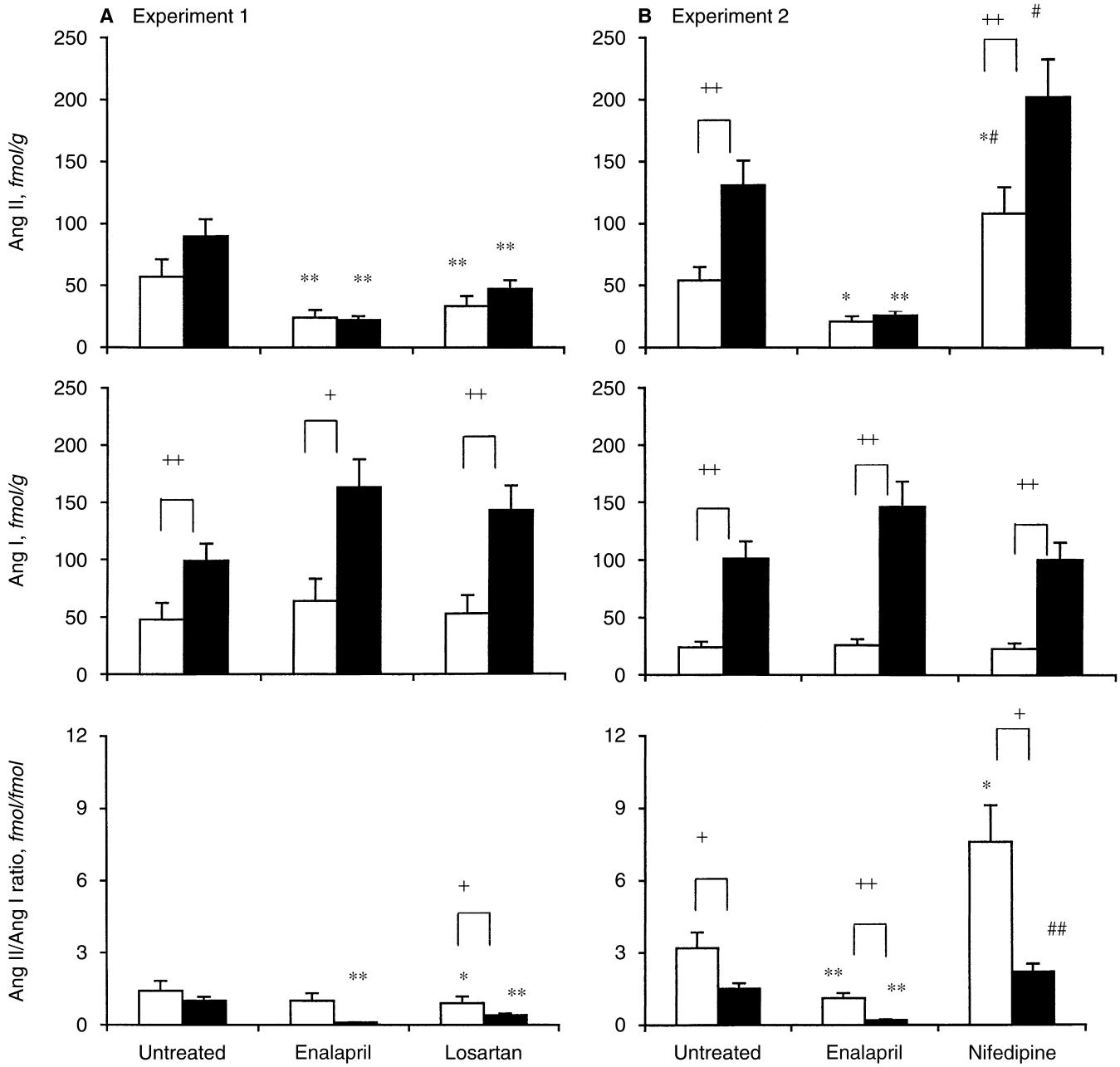

Fig. 2. Kidney angiotensin II (Ang II), Ang I and Ang II/Ang I ratio in experiments 1 and 2. Symbols are: (□) intact portion of the remnant kidney; (■) peri-infarct portion of the remnant kidney. $*P < 0.05$ vs. corresponding region of untreated rats; $**P < 0.01$ vs. corresponding region of untreated rats; $+P < 0.05$ peri-infarct vs. intact portion; $++P < 0.01$ peri-infarct vs. intact portion; $\#P < 0.05$ vs. enalapril treated rats; $\#\#P < 0.01$ vs. enalapril treated rats. Data are shown as mean ± SEM and $N = 7$ to 11 in each group.

that in comparison with enalapril, nifedipine caused a shift to the left and a steepening of the glomerulosclerosis score/blood pressure relationship that was associated with additional impairment of the already impaired renal autoregulation in the remnant kidney. At any given blood pressure, rats receiving nifedipine manifested more glomerular injury than rats receiving enalapril. Measurement of intrarenal Ang II levels in our current study provides a possible explanation for the different effects of these blood pressure-reducing agents. In contrast to enalapril and losartan, lowering the pressure with nifedipine was associated with an increase in Ang II levels in the intact functioning portion of the remnant kidney. The increase in Ang II levels in the intact portion of the remnant kidney was associated with an increase in the Ang II/Ang I ratio, suggesting that nifedipine may have increased renal ACE activity, thus contributing to increased Ang II levels in this region of the remnant

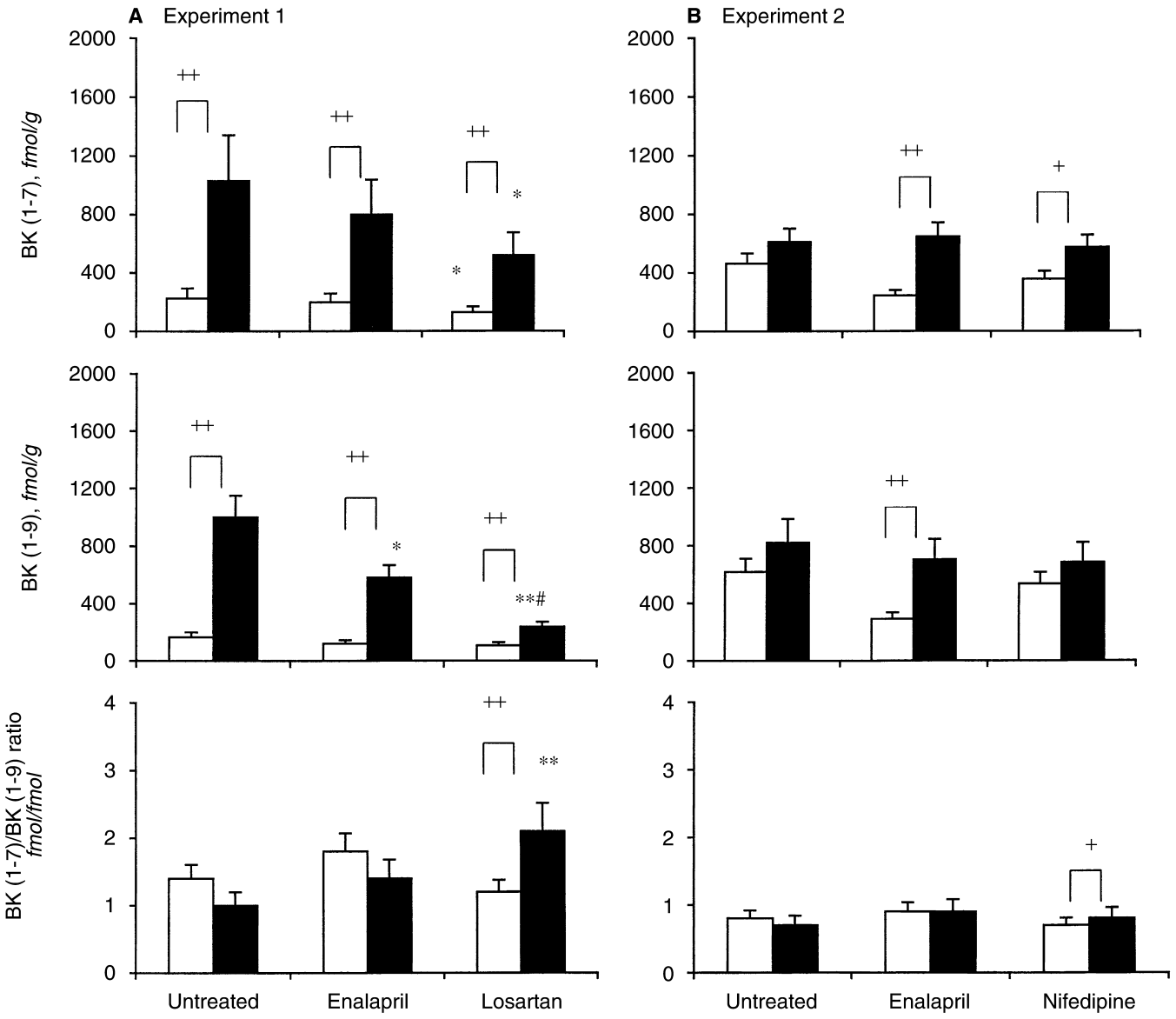

Fig. 3. Kidney bradykinin (1-7) [BK (1-7)], BK (1-9) and BK (1-7)/(1-9) ratio in experiments 1 and 2. Symbols are: (□) intact portion of the remnant kidney; (■) peri-infarct portion of the remnant kidney. $*P < 0.05$ vs. corresponding region of untreated rats; $**P < 0.01$ vs. corresponding region of untreated rats; $+P < 0.05$ peri-infarct vs. intact portion; $++P < 0.01$ peri-infarct vs. intact portion; $\#P < 0.05$ vs. enalapril treated rats. Data are shown as mean ± SEM and $N = 7$ to 11 in each group.

kidney. The increase in PRA with nifedipine treatment may have been a response to the fall in blood pressure, leading to increased renin secretion from the ischemic portions of the remnant kidney. However, we are unable to explain the lack of increase in plasma angiotensin peptide levels in nifedipine-treated rats. The levels of plasma angiotensin peptides are determined by both their rate of production and rate of degradation. We did not examine Ang II degradation in these experiments, and further experiments would be necessary to address whether changes in Ang II degradation contributed to the failure of plasma Ang II levels to rise with nifedipine.

In a previous study, we found that the remnant kidney model is associated with transient increases in peri-infarct kidney Ang II levels and plasma Ang II and aldosterone levels at two weeks [21]. However, plasma Ang II and aldosterone, as well as peri-infarct Ang II levels were not higher than in control rats at 5 and 7 weeks following renal ablation. Moreover, Ang II levels in the intact portion of the remnant kidney were not higher than in the kidney of control rats. This finding, along with the finding of Verhagen et al [22] of normal Ang II levels in the kidneys of rats made hypertensive by nitric oxide synthase inhibition, indicates that an increase

in intrarenal Ang II content above normal is not necessary for the development of injury in intact renal tissue in the setting of high blood pressure.

The remnant kidney model is complex and involves many pathological processes in addition to reduction in nephron number. The increase in blood pressure and development of glomerular injury are in general more severe when nephron number is reduced by partial infarction of the kidney than by excision of renal tissue [23, 24]. One potential mechanism is by promoting an increase in glomerular capillary pressure. Presumably increased intrarenal Ang II levels could account for the finding that glomerular pressure remains high when blood pressure in rats subjected to renal ablation is lowered by the "triple therapy" antihypertensive combination of reserpine, hydralazine and thiazide [11]. In addition to hemodynamic effects, Ang II may promote glomerulosclerosis by stimulating expression of transforming growth factor-β (TGF-β) and platelet-derived growth factor-β (PDGF-β) [25]. There is also evidence that Ang II may directly promote tubulointerstitial injury in the remnant kidney. Inflammatory phenomena such as macrophage and lymphocyte infiltration, together with tubulointerstitial myofibroblast transdifferentiation and tubulointerstitial cell proliferation, have been implicated in tubulointerstitial injury [26]. Recent studies show that increased Ang II levels can promote renal inflammation by stimulating monocyte and macrophage infiltration [27]. Gilbert et al reported that intrarenal renin and Ang II content are predominant in renal tubular epithelial cells of the remnant kidney at 12 weeks after renal ablation [28]. These authors suggest that renin and Ang II content in tubular epithelial cells may be pathogenically involved in the progressive tubulointerstitial injury of this condition. However, it is not known whether tubular epithelial expression of renin and Ang II occurs at the earlier time after renal ablation examined in the present study. Gilbert et al also noted that ACE inhibition prevents tubular epithelial expression of renin and Ang II, suggesting that renin and Ang II content in the renal tubular epithelium is secondary to Ang II-dependent pathogenic processes in the remnant kidney [28].

An interesting finding of the current study was that losartan as well as enalapril reduced intrarenal Ang II levels. The doses of enalapril and losartan used caused similar reductions in blood pressure and similar increases in PRA and plasma Ang I levels. Whereas converting enzyme inhibition reduced the plasma Ang II/Ang I ratio so that the increase in plasma Ang I level was not accompanied by an increase in Ang II level, AT_1 receptor blockade did not affect the plasma Ang II/Ang I ratio and the plasma Ang II level increased in parallel with the plasma Ang I level in rats given losartan. However, the pattern of changes in intrarenal angiotensin peptides was different from that in plasma. Both agents reduced renal Ang II levels in the peri-infarct and intact portions of the remnant kidney. The mechanism by which both ACE inhibition and AT_1 receptor blockade reduce intrarenal Ang II levels remains to be conclusively established. We previously showed that reduction in kidney Ang II levels with perindopril in normal rats was not accompanied by a rise in Ang I levels [29]. Furthermore, there was a reduction in kidney angiotensinogen levels suggesting that local consumption of angiotensinogen occurred due to a large increase in renin secretion. We therefore proposed that local consumption of angiotensinogen limits any increase in renal angiotensin peptide levels during perindopril administration [29]. We have shown previously that losartan reduced Ang II levels in the normal kidney [30], which may be due to a similar mechanism. Moreover, losartan may prevent renal uptake of Ang II by blockade of the AT_1 receptor as demonstrated in rats infused with an exogenous form of Ang II [31]. In addition, losartan reduced the Ang II/Ang I ratio in the remnant kidney, indicating that losartan may have reduced Ang I conversion to Ang II, possibly by reducing renal ACE activity. We previously observed that losartan administration reduced the renal Ang II/Ang I ratio in normal rats [30].

We previously showed marked increases in bradykinin peptide levels in the intact and peri-infarct portions of the remnant kidney [21]. In addition to reducing Ang II levels, losartan reduced bradykinin levels in the peri-infarct portion and enalapril reduced levels in the intact portion of the remnant kidney. We propose that the decrease in intrarenal bradykinin levels in response to losartan and enalapril was due to the suppression of the Ang II-dependent inflammatory processes by these drugs. There has been some debate as to the role of bradykinin in mediating the renoprotective actions of ACE inhibition, with reports that bradykinin type 2 (B2) receptor antagonists do not modify the effects of ACE inhibition [32]. Our demonstration that losartan and enalapril actually decrease intrarenal bradykinin levels provides an explanation for the failure of B2 receptor antagonism to modify the effects of ACE inhibition. The reduction in intrarenal bradykinin levels in response to enalapril was in contrast to our earlier studies in normal rats where inflammation was absent and ACE inhibition increased bradykinin levels by inhibiting bradykinin metabolism [18, 33]. We previously showed that losartan reduced intrarenal bradykinin levels in normal rats [30], a finding that suggests that intrarenal bradykinin levels are subject to tonic stimulation by Ang II acting through the AT_1 receptor. Our findings are in contrast to the studies of Siragy, de Gasparo and Carey, who found that AT_1 receptor antagonism increased immunoreactive bradykinin levels in microdialysate fluid from the rat kidney [34]. The difference between our data and those of Siragy

et al [34] may be due to our use of a different experimental model.

Our findings do not reveal the extent to which changes in intrarenal bradykinin levels contributed to the reduction of blood pressure and proteinuria observed with enalapril and losartan treatment. Bradykinin has many effects on the kidney, including the promotion of sodium excretion and prostaglandin and nitric oxide release [35]. Several lines of evidence suggest a role for bradykinin in mitigating hypertension. Pharmacological blockade of the B2 receptor and B2 receptor gene knockout exacerbates hypertension [36–39]. Thus, increased intrarenal bradykinin levels may act to limit the increase in blood pressure following renal ablation. Other actions of bradykinin, however, may have adverse effects on the remnant kidney. Bradykinin may contribute to remnant kidney injury by non-hemodynamic mechanisms including mesangial cell proliferation and collagen production [40]. Additional to B2 receptor-mediated processes, inflammation induces expression of the bradykinin type 1 (B1) receptor in the kidney, thus potentiating the inflammatory actions of bradykinin [41]. We have no information on the mechanism of production of bradykinin in the remnant kidney, but it is likely that inflammatory cells may contribute to intrarenal bradykinin levels in this model. Whereas the main sites of bradykinin formation in the normal kidney are likely to be within the vasculature and tubules, inflammatory cells may cause the generation of high levels of bradykinin within the interstitium of the remnant kidney where they may act to promote the inflammatory process. Further experiments are required to test whether reduction in inflammatory cells with RAAS blockers leads to decreased BK levels in the kidney.

In conclusion, we found that drugs such as enalapril and losartan, which block the actions of Ang II, reduce proteinuria, whereas nifedipine at a dose that produced an equivalent blood pressure-lowering effect increased proteinuria and intrarenal Ang II levels. In addition, we found that losartan and to a lesser extent enalapril, reduced intrarenal bradykinin levels. This study supports a primary role for Ang II in the pathogenesis of renal insufficiency in the remnant kidney model, and also indicates that the inflammatory actions of Ang II may be due in part to promotion of bradykinin formation.

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